An evaluation of Next Generation Sequencing for genetic diagnosis of the Primary Hyperoxalurias

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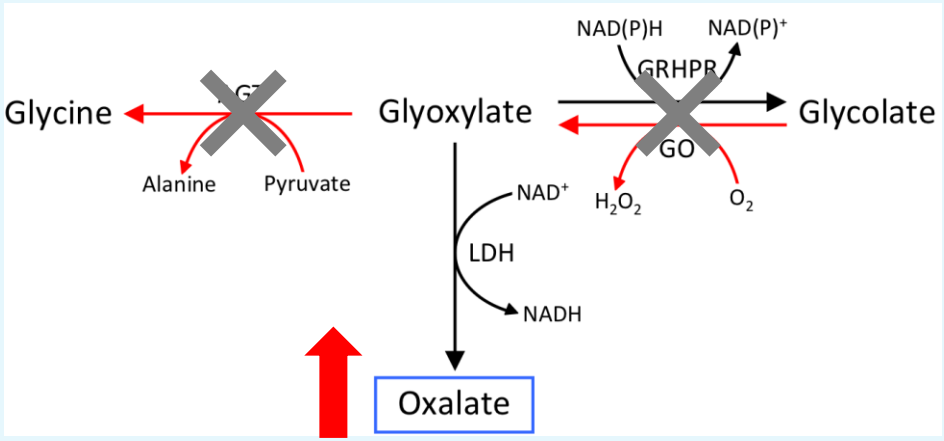
Primary Hyperoxaluria (PH)

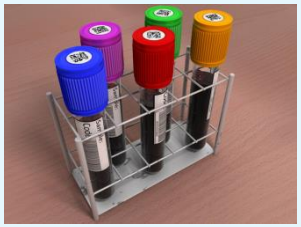
- Affects ~3 in 1 million individuals
- Caused by defects in glyoxylate metabolism
- Excess glyoxylate converted to oxalate
- Oxalate excess
  - Kidney stones
  - Renal failure
  - Kidney/liver transplant



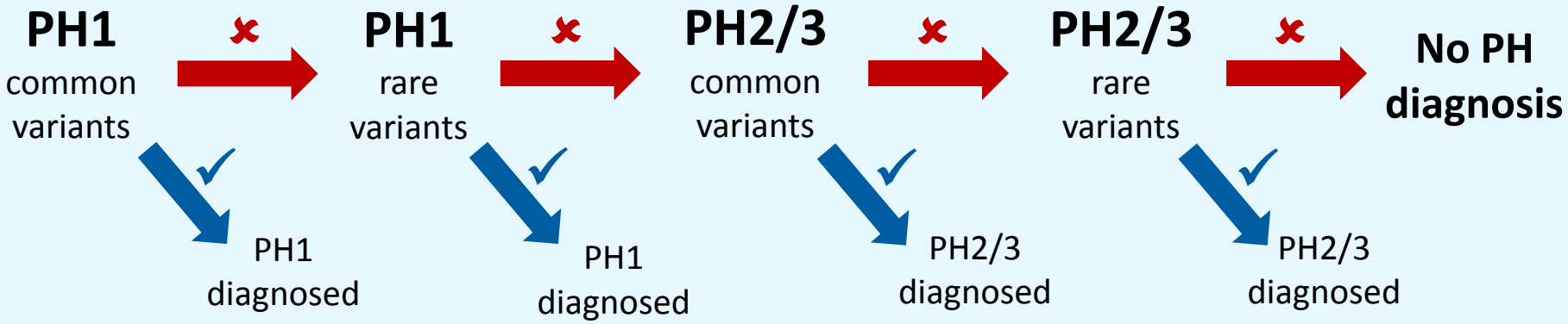
- Three associated genes
  - **PH1**: *AGXT*
  - **PH2**: *GRHPR*
  - **PH3**: *HOGA1*

Current laboratory diagnosis

- Biochemistry (blood and urine)
- Liver biopsy enzyme analysis
- Kidney stone analysis

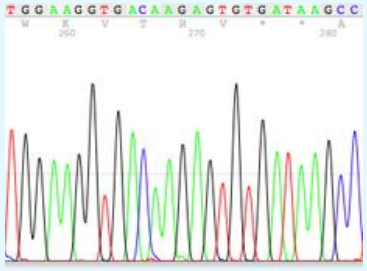


- Genetic testing to identify two disease causing mutations
- Requires one blood sample
  - Sequential diagnostic approach:



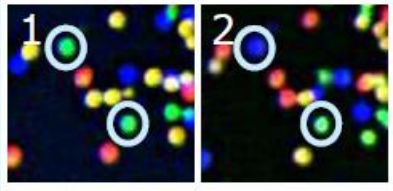
Sequencing Technologies

Sanger sequencing



- Traditional method for DNA sequencing
- Gene-by-gene analysis
- Widely used in UK for Clinical Diagnostics
- Labour-intensive, few samples analysed simultaneously

Next Generation Sequencing (NGS)



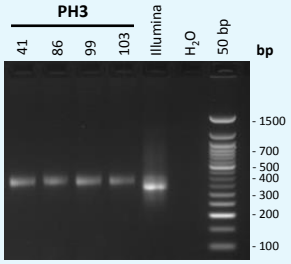
- Targeted methods e.g. for 3 x PH genes
- Illumina “TruSeq Custom Amplicon”
- Many samples and several genes analysed simultaneously
- Bioinformatic support required for data analysis

Sample analysis

- Genomic DNA from 90 patients previously diagnosed with PH
  (Provided by Dr Gill Rumsby, UCLH)

Day 1 **Prepare DNA samples**

Days 2-3 **TruSeq Custom Amplicon Protocol**



Days 4-5 **Determine DNA sequence using MiSeq**

Days 6-7 **Analyse data, identify mutations, report results**

Comparing Sanger and NGS results

96.7 % agreement between methods for PH-causing variants

PH1 **58 of 64 PH1 patients correctly diagnosed**

- 1 patient: no previous genetic diagnosis
- 1 patient: known large gene deletion
  - cannot be detected by NGS
- 4 patients: NGS gave one incorrect variant
  - poor quality data in sections of PH1 gene

PH2 **All 14 patients correctly diagnosed**

PH3 **All 12 patients correctly diagnosed**

Additional data from NGS method

- Mutations in two different PH genes for 3 PH1 patients
  - 2 PH1 variants and 1 PH3 variant
  - Patients diagnosed with PH1

    ➔ **Clinical impact?**

- Novel variants found in all 3 genes

    ➔ **Assess pathogenicity**

  - 4 likely disease-causing
  - 2 likely benign

Outcomes

- Targeted NGS assay for PH developed - 90 samples analysed
- 84 cases given genetic diagnosis of PH
- 96.7 % agreement with Sanger sequencing results
- 6 novel mutations identified
- Single assay could give diagnosis in 1 week

Use in Clinical Diagnostics?

- Expensive equipment
- Initial bioinformatics support vital
- Not suitable for large gene deletions
- Confirmation using traditional methods still necessary
- Cheaper and faster for analysis of several genes
- Additional information may be obtained

Acknowledgements

- Imperial College Healthcare NHS Trust
  - Dr Emma Walker

- Imperial College MRC Clinical Sciences Centre
  - Dr Jana Vandrovcova
  - Dr Michael Mueller
  - Prof Tim Aitman

- UCLH
  - Dr Gill Rumsby